

ORIGINAL ARTICLE

Effects of bovine serum concentrate, with or without supplemental micronutrients, on the growth, morbidity, and micronutrient status of young children in a low-income, peri-urban Guatemalan community

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Objective: To determine the effects of dietary supplements containing bovine serum concentrate (BSC, a source of immunoglobulins) and/or multiple micronutrients (MMN) on children's growth velocity, rates of common infections, and MN status. **Design:** Randomized, controlled, community-based intervention trial.

Setting: Low-income, peri-urban Guatemalan community.

Subjects: Children aged 6–7 months initially.

Interventions: Children received one of four maize-based dietary supplements daily for 8 months, containing: (1) BSC, (2) whey protein concentrate (WPC, control group), (3) WPC + MMN, or (4) BSC + MMN.

Results: There were no significant differences in growth or rates of morbidity by treatment group. Children who received MMN had lower rates of anemia and (in the group that received WPC + MMN) less of a decline in serum ferritin than those who did not, but there were no differences in other biochemical indicators of MN status by treatment group.

Conclusions: MMN supplementation reduced anemia and iron deficiency in this population, but the MMN content and source of protein in the supplements did not affect other indicators of MN status, growth or morbidity.

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Introduction

More than 250 million pre-school children in lower income countries are affected by nutritional stunting, defined as

length-for-age < -2 s.d. with respect to the international reference population median (De Onis *et al.*, 2000). In Guatemala, for example, nearly 50% of children less than 5 years of age have low height-for-age (Instituto Nacional de Estadística, Center for Disease Control 2003), and most of the height deficit of Guatemalan adults can be attributed to growth failure during early childhood (Martorell, 1995).

Inadequate dietary intakes and high rates of infections are believed to be the principal post-natal biological factors that limit children's growth, although the specific nutritional causes of growth stunting remain uncertain (Waterlow, 1994). Several reports indicate that particular aspects of dietary quality, especially micronutrient density and bio-availability, as well as the proportion of nutrients obtained

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from animal source foods may be important determinants of children's growth (Allen, 1994). An earlier trial in Guatemala found that supplementation with zinc alone increased the growth of stunted, but not of non-stunted, infants (Rivera *et al.*, 1998), findings which are consistent with the results of a meta-analysis of the effect of zinc supplementation on children's growth (Brown *et al.*, 2002). Other studies of multiple micronutrients (MMN) found positive effects of supplementation on growth among Mexican (Rivera *et al.*, 2001) and Vietnamese (Thu *et al.*, 1999; Hop and Berger, 2005) infants and among older children in Tanzania (Ash *et al.*, 2003), suggesting that deficiency of one or more of these micronutrients was limiting their physical development. Contrary to these results, multiple micronutrient (MMN) supplements did not affect the growth of children in other settings (Penny *et al.*, 2004; López de Romaña *et al.*, 2005; Smuts *et al.*, 2005; Untoro *et al.*, 2005), possibly because the study subjects were less growth restricted initially, they were not lacking in any of the specific micronutrients that are critical for growth, or the supplements that were provided were poorly absorbed.

Experimental trials in newly weaned farm animals have demonstrated that incorporation of spray-dried bovine or porcine blood plasma into their feeds increases their dietary intakes and produces greater rates of weight gain compared with control diets (Weaver *et al.*, 1995; Pierce *et al.*, 2005), possibly because the higher concentrations of immunoglobulins in the plasma-fortified products protected against infection. We therefore proposed that similar benefits might occur in young children in populations that experience high rates of diarrhea and other infections. Food-grade bovine serum concentrate (BSC) prepared from hygienically collected and processed blood is now available commercially as a spray-dried powder and for use in the preparation of processed foods. A preliminary clinical study found that BSC incorporated into an infant formula was well tolerated by a small group of young Peruvian children recovering from malnutrition (Lembcke *et al.*, 1997).

The present research was completed to determine whether dietary supplementation with MMN, BSC, or both would enhance the physical growth and micronutrient status of Guatemalan infants compared with a supplement containing whey protein and no added micronutrients. We hypothesized that infants who received a small, daily supplement of a maize-based pudding containing: (1) BSC, (2) BSC and MMN, or (3) whey protein concentrate (WPC) and MMN for 6–8 months would have increased linear growth and accrual of body weight compared with those who received the same pudding supplemented only with WPC. We further postulated that the children who received the supplements containing MMN would have higher levels of selected biochemical indicators of micronutrient status at the end of the period of supplementation, regardless of the protein source. Finally, we assumed that these outcomes would be mediated at least partially through reductions in infectious diseases in the groups that received BSC and/or

MMN, although the available sample was only sufficient to permit detection of fairly large differences in morbidity rates.

Methods

Study design

The study was designed as a randomized, double-masked, community-based trial of maize-based dietary supplements that contained either WPC (control group), BSC, WPC + MMN, or BSC + MMN in a small amount of a pudding that was provided daily (6 days per week under field worker supervision, with one dose left with the caregiver for administration on the seventh day).

Study site, subjects, and randomization procedures

The study was conducted in nine low-income neighborhoods ('asentamientos') on the outskirts of Guatemala City. A census was completed initially and 5 and 10 months later to identify all infants <5 months of age who were eligible to participate in the study. Children were excluded from consideration if: (1) they were severely malnourished (defined as length-for-age <−3z or weight-for-length <−2z with respect to World Health Organization (WHO)/NCHS/CDC (National Center for Health Statistics/Centers for Disease Control) reference data (US National Center for Health Statistics, 1977); (2) they had evidence of congenital abnormalities that might interfere with growth or predispose to infection; or (3) their parents planned to move from the study community within the next few months or did not consent to participate. The study protocol was approved by the institutional review boards of the University of California, Davis and the Institute of Nutrition of Central America and Panama; a parent of each child provided written, informed consent.

Previous observations in the study community indicated that more than half of the infants were anemic (defined as altitude-corrected hemoglobin <10.3 g/dl, corresponding to a measured hemoglobin <11.0 g/dl) at 6 months of age. Therefore, all children who were enrolled in the current study were given daily supplements of 10 mg iron (as ferric ammonium citrate) as a preventive measure during the period from 4 to 5 months of age. At 5 months of age, hemoglobin levels were measured in capillary blood by using a portable hemoglobinometer (HemoCue Inc., Mission Viejo, CA, USA), and any child who was anemic was given 20 mg iron per day during the following 1–2 months. Children whose hemoglobin was still <11.0 g/dl at 7 months were dropped from the study and referred for further evaluation and treatment.

When the children became 5 months of age, disease surveillance was initiated by a field worker, who visited the home 6 days per week to inquire about specific symptoms of illness during the previous 1 or 2 days, as described below. Once the children became 6–7 months of age, those who

were eligible for the study were stratified by sex and randomly assigned to one of the 12 letter codes, using a block randomization scheme, with block length of 12. Each of the code letters corresponded to one of the four treatment groups. Baseline anthropometric and biochemical assessments were completed before treatments were initiated. The identity of the individual treatments remained masked until all data collection, laboratory assays, and preliminary analyses of data were completed.

Supplements

Children <12 months of age were provided with ~14.2 g (dry weight) of the supplements each day, using a pre-calibrated measuring spoon. The supplements were composed of maize flour, maltodextrins (corn syrup solids), sugar, flavoring agents, WPC or BSC, and either a vitamin/mineral supplement or additional maize flour (Table 1). In the two groups that received BSC, immunoglobulin G (IgG) accounted for approximately 22% (0.68 g) of the 3.1 g of BSC (NutraGammax 20; LG Laboratories, Ames, IA, USA) included in each daily supplement. The MMN supplement contained one US recommended dietary allowances (RDA) (Food and Nutrition Board, National Research Council 1989) for 1-year old children of each of the nutrients that were included, except for calcium and phosphorus, which were present at a level of approximately 0.3 RDA (Table 2). When children reached 12 months of age, they received 1.5 times the aforementioned doses of each supplement (i.e., ~21.3 g/day dry weight). The ingredients of each supplement were combined as dry agglomerates to enhance their ultimate dispersion in aqueous mixtures, and were stored in sealed, coded cans before labeling with the individual children's names and distribution to their respective homes. The composition and identity of the supplements were independently confirmed on several occasions by measuring the amounts of IgG and selected micronutrients in the mixtures.

Table 1 Composition of dry supplements, by treatment group (amount of ingredient, in grams, per daily dose of supplement, infants 6–11 months^a)

Ingredient	Treatment group			
	WPC	MMN+WPC	BSC	MMN+BSC
Maize flour	2.9	2.5	2.9	2.5
Maltodextrin (corn syrup solids)	1.6	1.6	1.6	1.6
Sugar	3.8	3.8	3.8	3.8
Lecithin, flavoring agents	0.9	0.9	0.9	0.9
Bovine serum concentrate	0	0	3.1	3.1
Whey protein concentrate	5.0	5.0	1.9	1.9
Micronutrient mix	0	0.4	0	0.4

Abbreviations: BSC, bovine serum concentrate; MMN, multiple micronutrients; WPC, whey protein concentrate.

Supplements prepared as semi-solid 'pudding' by adding ~20–30 ml of water.

^aChildren ≥12 months received 1.5 times this dose.

Table 2 Composition of micronutrient mix provided to MMN groups (amount of ingredient per daily dose of supplement, infants 6–11 months^a)

Nutrient (compound)	Amount
Vitamin A (retinyl palmitate)	400 µg RE
Vitamin B ₁ (thiamin mononitrate)	0.4 mg
Vitamin B ₂ (riboflavin)	0.5 mg
Niacin	0.6 mg
Vitamin B ₆ (pyridoxine HCl)	0.6 mg
Vitamin B ₁₂ (cyanocobalamin)	0.5 µg
Vitamin C (ascorbic acid)	35 mg
Vitamin D ₃ (cholecalciferol)	400 IU
Folic acid	35 µg
Iron (as electrolytic iron)	10 mg
Zinc (as zinc sulfate)	5 mg
Iodine (as potassium iodide)	50 µg
Selenium (as sodium selenite)	15 µg

Abbreviations: MMN, multiple micronutrients'; RE, retinal equivalents.

^aChildren ≥12 months received 1.5 times this dose.

Field workers visited each home daily (6 days per week) and added 20 or 30 ml of clean water to the appropriate dose of dry supplement to make a pudding-like mixture, which was fed to the child by spoon. The field worker observed and recorded the amount consumed as none, 25, 50, 75, or 100% of the assigned portion. On Saturdays, a dose of the dry supplement was left with the child caregiver for administration on Sunday, and on days when the child was not present in the home or was otherwise unavailable (~15% of days), the field worker prepared the day's dose and left it with the caregiver for administration shortly thereafter. In these latter cases, the amount consumed was recorded the following day.

Anthropometry

The children's anthropometric status was assessed at baseline and monthly thereafter in their own homes, using standard techniques (Lohman *et al.*, 1988). Measurements were taken by one of two anthropometrists who were periodically standardized against the field supervisor. Body weight was measured to the nearest 5 g, using a frequently standardized electronic balance (Tanita Model 1583; Tokyo, Japan). Recumbent length was measured to the nearest 0.1 cm, using a wooden length board (Shorr, Columbia, MD, USA). Mid-upper arm circumference (MUAC) was measured to the nearest 0.1 cm, using a fiberglass tape. Intra- and inter-observer measurement errors were assessed periodically by repeated measurement of a separate group of children, and the combined measurement error was ±0.23 cm (coefficient of variation, CV = ±0.3%) for length and ±0.21 cm (CV = ±1.4%) for MUAC.

Morbidity

Morbidity from common childhood illnesses was assessed by the same field worker who observed the daily administration

of the supplements. The field worker questioned the child's primary caregiver systematically to elicit information on stool number and consistency, and the presence of specific symptoms of respiratory and other infections, using a modified version of a previously developed questionnaire (López de Romaña *et al.*, 1989). Diarrhea was defined as excretion of ≥ 3 loose or liquid bowel movements per day, and an episode of illness was considered to have ended when the stool pattern did not satisfy these criteria for at least 2 days. Diarrhea was also defined using a more demanding cut-off of ≥ 4 loose or liquid bowel movements per day to see if this affected the conclusions. An episode of diarrhea was designated as severe if any one of the following occurred on any day of the episode: ≥ 6 liquid or semi-liquid stools, fever, vomiting, blood or mucous in the stool, or dehydration. Diarrhea was defined as persistent if it lasted ≥ 14 days. Upper respiratory infection was defined as the presence of cough associated with purulent nasal discharge; while lower respiratory infection was defined as the presence of cough and age-specific elevated respiratory rate (> 50 /min for infants < 12 months of age and > 40 /min for older children), as per recommendations of the World Health Organization (2000). Incidence was defined as the number of new episodes of each disease per 100 days at risk, and prevalence was defined as the percent of days of observation on which the disease was present.

Dietary assessment

Dietary intakes were assessed at baseline and every 3 months thereafter by means of direct weighing of all foods and beverages consumed during daytime observations, including test weighing of breast milk intake, and recall of any foods consumed at night. Breast milk consumption during the 10-h daytime observations was extrapolated to 24 h, by multiplying the daytime intakes by a correction factor, determined during 24-h observations completed in a subsample of 16 mother–infant pairs. The macronutrient contents of breast milk were imputed, using values published by the WHO (Brown *et al.*, 1998). Energy and nutrient intakes from complementary foods were calculated by using the food composition table published by the Institute of Nutrition of Central America and Panama (INCAP/OPS, 1996) and additional data for the zinc (US Department of Agriculture, Agricultural Research Service 2002) and phytate (Calloway *et al.*, 2005) contents of foods.

Biochemical assessment

A sample of venous blood was obtained at baseline and at the end of the period of supplementation for measurement of hemoglobin and serum ferritin, retinol, and vitamin E, and plasma zinc and copper concentrations. The samples were generally obtained in the morning, but were not standardized in relation to recent meals or breast-feeding. When the children were acutely ill with diarrhea or fever on the day of scheduled blood drawing, the sample collection was post-

poned until the symptoms resolved. Hemoglobin was analyzed in the field using the HemoCue device, and results were corrected for altitude to determine the rates of anemia (Dirren *et al.*, 1994). The remainder of the blood samples was centrifuged and aliquoted in the field office before being transported to INCAP in cold boxes for subsequent storage at -17°C and shipment to the US on dry ice for analysis at UC Davis.

Serum ferritin was determined by enzyme immunoassay (Spectro Ferritin, Ramco Laboratories, Inc., Houston, TX, USA); and retinol and vitamin E were measured by reverse-phase high-pressure liquid chromatography (Bieri *et al.*, 1979), using a Shimadzu Class VP HPLC (Shimadzu, Columbia, MD, USA) equipped with a photo-diode array detector. Aliquots of plasma for trace element analysis were stored frozen in mineral-free, colorless plastic vials. For trace element analyses, 200 μl of the plasma was combined with 2.2 ml of 1N Ultrex II ultrapure HNO_3 (JT Baker Inc., Phillipsburg, NJ, USA), allowed to stand overnight at 4°C , and then centrifuged. The supernatants were analyzed for trace element concentrations, using a Tracescan inductively-coupled-plasma atomic-emission spectrometer (ICP-AES; Thermo Jarrell Ash; Spectrocell Inc., Oreland, PA, USA). All samples from the same child were measured in a single analytic run, and compared with appropriate ultrapure acid blanks, pooled plasma samples and ICP standards (QC-21 Spex, Fisher Scientific; Springfield, NJ, USA). All materials and procedures were checked to rule out possible zinc contamination.

Socioeconomic status

Socioeconomic status (SES) was assessed at the beginning of the study by parental interview and observation of the home. Information was collected on household composition; parental age, education and occupation; housing quality; and selected material possessions. The housing quality score was calculated as the sum of the floor, wall, and roof quality scores, each expressed on an ordinal scale of 0–1. The possessions score was the number of the following items owned by the family: radio, television, refrigerator, wardrobe, bed, iron, automobile, and bicycle.

Sample size

The required sample size was estimated for the primary outcomes of linear growth and weight gain during the planned 8-month period of supplementation. Using longitudinal data from previous studies in Guatemala (Habicht *et al.*, 1995), a sample size of 43 per group was estimated to be sufficient to permit detection of a 0.85 cm main effect in linear growth and a 0.30 kg main effect in weight gain among treatment groups, using a two-factor analysis of variance (ANOVA), considering a type-I error of 0.05 and power of 0.80. These detectable differences are less than half of the originally expected shortfall in growth of Guatemalan children compared with international reference data during

this age interval. The desired enrollment was set at a minimum of 54 children per group to allow for an estimated 25% rate of attrition.

Statistical analysis

Descriptive statistics (mean, standard deviation, range, skewness) were calculated for all outcome variables. Cubic polynomial interpolation was used to estimate each child's weight, length, and arm circumference at exact monthly intervals from the start of supplementation. Outcome variables were transformed, if necessary, to create approximately normal distributions with homogeneity of variance among groups. Outcomes of treatment were compared by using analysis of covariance, which included presence or absence of MMN and BSC as main effects and the following covariates: child demographics (sex and age at enrollment), baseline anthropometry (weight and length), baseline morbidity rates (prevalence of diarrhea, fever, and upper respiratory infection during the month before initiation of supplementation), baseline iron and zinc status (initial serum ferritin and plasma zinc concentrations), breastfeeding status, maternal factors (age, height, hemoglobin concentration, and level of education), household SES variables (housing quality and possessions scores), and, where appropriate, the baseline value of the outcome variable. Interaction terms included two- and three-factor interactions involving MMN and BSC with initial weight and length, baseline diarrhea rate, initial iron and zinc status, breastfeeding status, and baseline value of the outcome variable, where appropriate. Nonsignificant ($P > 0.10$) interactions and main effects ($P > 0.05$) were removed in a hierarchical fashion, with the exception that the intervention variables (i.e., treatment groups) were forced into the model. Morbidity variables were weighted by number of days of observation; children with less than 60 days of morbidity observations were not included in these latter analyses.

Results

Enrollment and completion

A total of 315 children from nine neighborhoods were potentially eligible for the study and were included in the initial period of iron supplementation, as shown in the profile of study subjects (Figure 1). Of these, 56 were not included in the intervention trial because the child's mother began to work outside the home or moved to another area (18%); the child still had low hemoglobin despite 2 months of iron supplementation (13%); the parents refused blood collection (12%) or dietary observations (11%); the child surpassed the age limit for enrollment (5%); or the parents declined to participate without providing a specific reason (41%). Thus, a total of 259 children were allocated to treatment group, of whom 225 (86.9%) completed ≥ 60 days of observation, 184 (71.0%) completed ≥ 180 days of

observation, and 132 (51.0%) finished the full 8 months of observation. There were no significant differences in the number of dropouts by treatment group at any time point. The growth and morbidity data are presented for those children who completed 8 months of observation to be consistent with final biochemical results, most of which were obtained after 8 months of supplementation. We also analyzed the growth increments for those children remaining in the study after 2, 4, and 6 months of supplementation, and we found that the conclusions drawn from the group-wise comparisons at earlier time points did not differ from those drawn from the children who completed all 8 months of observation. Likewise, we found no differences between the children who completed 8 months of observation and those who remained in the study for less time with regard to supplement intake, prevalence, or incidence of morbidity, and breastfeeding status.

Baseline characteristics

Baseline anthropometric status, rates of morbidity from common infections during the month before initiation of treatment, rates of breast-feeding, and indicators of socioeconomic status did not differ significantly by treatment group (Table 3). The children's mean age at the time of initiation of supplementation was 6.2 ± 0.3 months. In total, 83% of the infants were breastfeeding at baseline, and more than two-thirds were still breastfeeding at 12 months of age. Mean daily total energy intakes at baseline ranged from 87 to 89 kcal/kg body weight/day ($0.36\text{--}0.37$ MJ/kg BW/day) in the different treatment groups, of which an estimated $58 \pm 37\%$ was provided by breast milk. The mean dietary intakes of iron and zinc were less than 2 mg/day, and the mean intakes of vitamin A ranged from 507 to 590 μg retinol activity equivalents (RAE)/day. There were no significant differences in baseline nutrient intakes by treatment group. Mean phytate intakes ranged from 28 to 60 mg, and were lower in the MMN + BSC group ($P < 0.05$), although mean phytate:zinc molar ratios were low in all groups and did not differ by study group.

Mean baseline hemoglobin levels ranged from 11.4 to 11.6 g/dl in the different treatment groups. Mean serum ferritin concentrations ranged from 46.8 to 62.1 $\mu\text{g/l}$, and $< 4\%$ of infants had low serum ferritin values ($< 10 \mu\text{g/l}$). Mean serum retinol concentrations ranged from 23.1 to 25.4 $\mu\text{g/dl}$ initially, and 34.6% of children had serum retinol values $< 20 \mu\text{g/dl}$. Mean plasma zinc concentrations ranged from 64.8 to 70.7 $\mu\text{g/dl}$, and more than half of the children had plasma zinc values $< 65 \mu\text{g/dl}$. There were no significant differences among treatment groups at baseline for any of the micronutrient status indicators that were measured.

Consumption of supplements

According to the field workers' observations at the time of their daily home visits, at least some portion of the

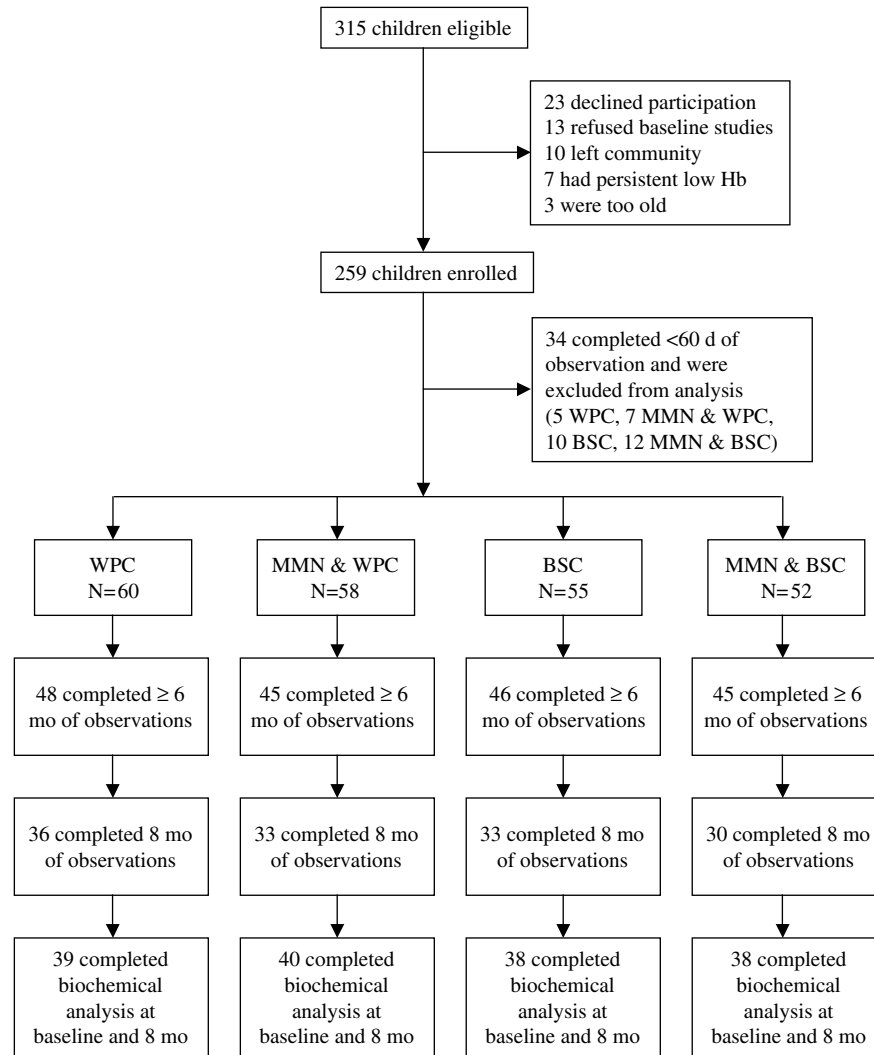


Figure 1 Profile of study subjects.

supplements (i.e., $\geq 25\%$ of the assigned amount) was consumed on $>98\%$ of study days, and this did not differ by treatment group ($P=0.16$). On days when at least some of the supplement was consumed, the average amount consumed was $\sim 80\%$ of the assigned dose (Table 4). The children who received supplements containing BSC consumed $\sim 12\%$ less of the amount offered than those who received mixtures containing WPC (Table 4).

Growth

Overall, the children gained approximately 8 cm during the 8-month period of observation (from approximately 6–14 months of age), which is $\sim 75\%$ of expected linear growth of North American children in this age range (US National

Center for Health Statistics, 1977). The study subjects gained about 1.8 kg, which is $\sim 63\%$ of expected weight gain. There were no significant differences in growth increments (including change in MUAC) by treatment group during the period of supplementation (Figure 2), controlling for initial anthropometric status, sex, and age at the beginning of supplementation. Controlling for additional covariates, such as feeding practices, maternal characteristics, socio-economic variables, and initial pre-supplementation morbidity rates did not affect the conclusions of these group-wise comparisons. Because of the high attrition rate during the course of the study, we also analyzed growth increments from baseline to 2 months, baseline to 4 months, and baseline to 6 months. In all cases, there were no significant treatment group-related differences in any of the growth indicators.

Table 3 Baseline characteristics of subjects included in at least one outcome assessment, by treatment group (mean \pm s.d. or %)

Variable	Group				P-value ^a
	WPC (N = 60)	MMN + WPC (N = 58)	BSC (N = 55)	MMN + BSC (N = 52)	
Male/female	29/31	27/31	27/28	26/26	0.99
Age at start of supplementation (months)	6.7 \pm 0.4	6.8 \pm 0.4	6.8 \pm 0.4	6.8 \pm 0.4	0.57
<i>Infant anthropometry at the start of supplementation</i>					
Weight (kg)	6.91 \pm 0.93	6.87 \pm 0.77	6.99 \pm 0.88	6.89 \pm 0.79	0.89
Length (cm)	64.1 \pm 2.5	63.9 \pm 2.2	64.1 \pm 2.4	64.1 \pm 2.0	0.93
Arm circumference (cm)	13.6 \pm 1.1	13.5 \pm 1.0	13.9 \pm 1.0	13.7 \pm 0.9	0.32
Weight-for-age (z)	-1.03 \pm 0.96	-1.10 \pm 0.81	-1.01 \pm 0.89	-1.09 \pm 0.76	0.92
Length-for-age (z)	-1.45 \pm 0.96	-1.59 \pm 0.74	-1.55 \pm 0.80	-1.53 \pm 0.63	0.82
Weight-for-length (z)	0.14 \pm 0.84	0.21 \pm 0.66	0.29 \pm 0.86	0.15 \pm 0.78	0.75
<i>Infant morbidity during the 30 days before supplementation</i>					
Prevalence of diarrhea (\geq 3 liquid or semi-liquid stools) (%)	9.1 \pm 13.8	17.3 \pm 23.5	9.8 \pm 15.9	13.2 \pm 22.5	0.17
<i>Infant nutrient intakes</i>					
Total energy (kcal/kg/day)	87.0 \pm 18.8	88.1 \pm 24.5	86.1 \pm 20.3	85.7 \pm 18.1	0.93
Energy from breast milk (kcal/kg/day)	54.3 \pm 30.5	50.2 \pm 32.8	52.7 \pm 30.2	48.6 \pm 33.9	0.79
Iron (mg/day)	1.3 \pm 1.4	1.5 \pm 2.5	1.2 \pm 1.1	1.0 \pm 1.1	0.50
Zinc (mg/day)	1.6 \pm 1.0	1.7 \pm 1.1	1.6 \pm 0.9	1.7 \pm 1.0	0.92
Vitamin A (μ g RAE/day)	530 \pm 221	535 \pm 255	565 \pm 244	508 \pm 198	0.64
Total phytate (mg/day)	44.2 \pm 70.5	59.0 \pm 81.5	52.4 \pm 73.6	28.7 \pm 37.3	0.13
Phytate:zinc molar ratio	2.6 \pm 3.7	3.2 \pm 3.9	2.9 \pm 4.2	1.7 \pm 1.9	0.19
<i>Infant biochemistry</i>					
<i>Hemoglobin (g/dl)</i>					
n	60	58	55	52	
mean \pm s.d.	11.6 \pm 0.7	11.5 \pm 0.6	11.6 \pm 0.6	11.5 \pm 0.6	0.46
<i>Serum ferritin (μg/l)</i>					
n	34	36	32	30	
mean \pm s.d.	51.0 \pm 49.4	46.8 \pm 44.3	53.1 \pm 40.3	62.1 \pm 50.2	0.48
% < 12 μ g/l	0.0	5.6	3.1	6.7	0.50
<i>Serum retinol (μg/dl)</i>					
n	31	36	31	36	
mean \pm s.d.	23.1 \pm 6.4	23.8 \pm 11.0	25.7 \pm 7.8	25.7 \pm 10.2	0.47
% < 20 μ g/dl	32.3	41.7	16.1	38.9	0.12
<i>Vitamin E (mg/dl)</i>					
n	31	36	31	36	
mean \pm s.d.	0.41 \pm 0.20	0.48 \pm 0.26	0.46 \pm 0.29	0.40 \pm 0.23	0.47
% < 0.5 mg/dl	71.0	58.3	64.5	69.4	0.68
<i>Plasma zinc (μg/dl)</i>					
n	33	39	34	34	
Mean \pm s.d.	69.9 \pm 19.8	66.2 \pm 15.6	70.1 \pm 19.8	65.7 \pm 19.8	0.64
% < 65 μ g/dl	48.5	53.8	50.0	50.0	0.97
<i>Plasma copper (μg/dl)</i>					
n	33	39	34	34	
Mean \pm s.d.	137 \pm 39	138 \pm 34	132 \pm 40	132 \pm 37	0.85
<i>Maternal nutrition and household socioeconomic variables</i>					
Maternal age (years)	24.3 \pm 5.6	25.2 \pm 6.0	25.4 \pm 6.0	25.9 \pm 6.6	0.57
Maternal education (years)	3.8 \pm 3.1	4.0 \pm 2.8	4.5 \pm 2.8	4.4 \pm 3.2	0.49
Housing quality score ^b	0.90 \pm 0.25	0.94 \pm 0.26	0.94 \pm 0.27	0.91 \pm 0.30	0.79
Possessions score ^b	4.6 \pm 1.5	4.5 \pm 1.7	4.9 \pm 1.5	4.2 \pm 1.8	0.26

Abbreviations: ANOVA, analysis of variance; BSC, bovine serum concentrate; CDC, centers for disease control; MMN, multiple micronutrients; NCHS, national center for health statistics; WPC, whey protein concentrate; RAE, retinol activity equivalents.

^aSignificance of group-wise comparisons (ANOVA for continuous variables, Kruskal-Wallis for categorical variables).

^bHousing quality score was calculated as the sum of floor, wall, and roof quality, each expressed on a scale of 0–1, with higher scores indicating better quality. Possessions score was calculated as the sum of specific items owned by the family.

Morbidity

All children who received supplementation for at least 60 days were included in the analyses of morbidity. Table 5

Table 4 Amount of supplement consumed (g/day), by age and treatment group^a

Age	Treatment group			
	WPC	WPC + MMN	BSC	BSC + MMN
< 12 mo	12.2 ± 2.1 ^a	12.2 ± 2.2 ^a	10.7 ± 2.4 ^b	10.6 ± 2.6 ^b
≥ 12 mo	19.1 ± 3.3 ^c	17.8 ± 3.5 ^c	16.6 ± 4.5 ^d	16.1 ± 3.8 ^d

Abbreviations: ANOVA, analysis of variance; BSC, bovine serum concentrate; MMN, multiple micronutrients; WPC, whey protein concentrate.

^aMeans are from >98% of days when at least some of the supplement was consumed. Values with different superscripts are significantly different, $P < 0.0001$ (3-way repeated measures ANOVA with main effects BSC treatment, MMN treatment, age, and their interactions).

shows the mean prevalence and incidence of specific types of morbidity during the period of supplementation by treatment group. The mean prevalence of diarrhea ranged from 10.4 to 13.5%, and did not differ significantly by treatment group, either before or after controlling for sex, age at initiation of supplementation, initial diarrhea rates during the pre-supplementation observation period, breast-feeding practices, maternal characteristics, initial plasma zinc and serum ferritin concentrations, and socioeconomic variables. Likewise, there were no significant differences in the incidence of all diarrhea combined, or in the prevalence or incidence of severe or persistent diarrhea by treatment group. The diarrhea rates displayed in the table are based on the definition of ≥3 liquid or semi-liquid stools per day. Results of group-wise comparisons did not change when a cutoff of ≥4 liquid or semi-liquid stools per day was used instead. There were also no significant differences by

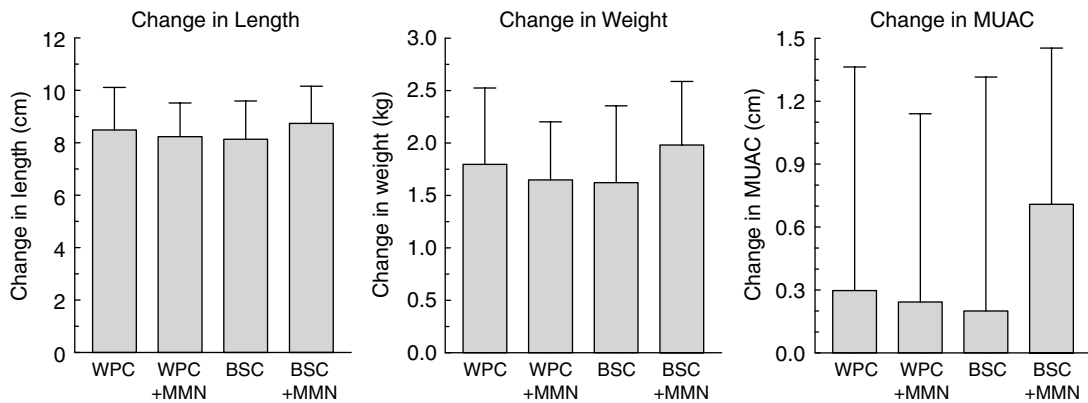


Figure 2 Mean (s.d.) change in final length, weight, and mid-upper arm circumference (MUAC), by treatment group (WPC = whey protein concentrate, BSC = bovine serum concentrate, MMN = multiple micronutrients). No significant differences by treatment group.

Table 5 Prevalence and incidence of morbidity during period of supplementation, by treatment group (mean + s.d.)^a

Variable	Treatment group				P-value ANOVA ^b
	WPC (n = 60)	WPC + MMN (n = 58)	BSC (n = 55)	BSC + MMN (n = 55)	
Prevalence of diarrhea (%)	10.2 ± 8.4	13.5 ± 13.2	10.4 ± 9.6	11.9 ± 13.0	0.98
Incidence of diarrhea	3.6 ± 2.6	5.1 ± 4.8	3.8 ± 3.0	3.9 ± 4.1	0.76
Incidence of severe diarrhea	1.9 ± 2.0	2.7 ± 3.0	1.9 ± 1.8	2.1 ± 2.5	0.96
Incidence of persistent diarrhea	0.13 ± 0.34	0.19 ± 0.58	0.03 ± 0.19	0.15 ± 0.40	0.19 ^c
Prevalence of fever (%)	9.4 ± 7.3	9.0 ± 6.2	10.5 ± 7.8	8.8 ± 6.8	0.86
Incidence of fever	3.9 ± 2.9	3.6 ± 2.2	3.9 ± 2.7	3.6 ± 2.5	0.78
Prevalence of URI (%)	7.1 ± 10.2	6.0 ± 8.3	7.1 ± 9.6	6.9 ± 9.5	0.96
Incidence of URI	1.4 ± 1.6	1.3 ± 1.7	1.4 ± 1.6	1.6 ± 2.3	0.53
Prevalence of LRI (%)	1.2 ± 3.4	1.1 ± 2.7	1.2 ± 2.7	1.4 ± 2.9	0.85
Incidence of LRI	0.6 ± 1.6	0.5 ± 1.0	0.5 ± 0.9	0.7 ± 1.4	0.95

Abbreviations: ANOVA, analysis of variance; BSC, bovine serum concentrate; LRI, lower respiratory infections; MMN, multiple micronutrients; URI, upper respiratory infections; WPC, whey protein concentrate.

^aAnalyses include children with at least 60 days of observation after initiation of supplementation. Incidence is defined as new episodes per 100 days at risk; prevalence is defined as percent of days ill. Diarrhea is defined as ≥3 liquid or semi-liquid stools per day. Persistent diarrhea is defined as diarrhea with ≥6 liquid or semi-liquid stools, fever, vomiting, blood or mucous in the stool, or dehydration. Persistent diarrhea is defined as an episode of diarrhea lasting ≥14 days.

^bANOVA, controlling for pretreatment morbidity rates. Logarithm of morbidity rate was used in all analyses, except incidence of persistent diarrhea, as explained in footnote c.

^cLogistic regression was used for this variable. (Because of the low incidence of persistent diarrhea, logarithmic transformation was not appropriate.)

Table 6 Biochemical indicators of micronutrient status following 8-month period of supplementation, by treatment group

Variable	Treatment group				P-value ^a
	WPC	MMN + WPC	BSC	MMN + BSC	
Hemoglobin					
<i>n</i>	39	37	37	36	
Mean \pm s.d. change from baseline	-0.26 \pm 0.88	-0.06 \pm 1.00	-0.36 \pm 1.25	0.15 \pm 0.93	0.096 ^b
% < 11.0 g/dl	37.1	22.1	37.4	17.0	0.11 ^b
Serum ferritin (μg/l)^c					
<i>n</i>	33	36	30	30	
Mean \pm s.d. change from baseline	-33.9 \pm 44.7 ^{ab}	-19.4 \pm 32.9 ^b	-34.4 \pm 35.8 ^{ab}	-46.6 \pm 49.0 ^a	0.034 ^d
% < 12 μ g/l	51.5	27.8	43.3	43.3	0.25
Plasma zinc (μg/dl)					
<i>n</i>	26	29	24	27	
Mean \pm s.d. change from baseline	-2.0 \pm 24.2	-0.6 \pm 20.1	4.5 \pm 15.9	2.0 \pm 18.7	0.84
% < 65 μ g/l	46.2	37.9	50.0	44.4	0.86
Plasma copper (μg/dl)					
<i>n</i>	26	29	24	27	0.58
Mean \pm s.d. change from baseline	14.4 \pm 38.2	13.9 \pm 35.4	27.9 \pm 28.9	23.5 \pm 38.2	
Serum retinol^f (μg/dl)					
<i>n</i>	25	29	24	25	
Mean \pm s.d. change from baseline	6.4 \pm 11.1	5.8 \pm 8.1	3.4 \pm 0.3	2.9 \pm 11.4	0.65
% < 20 μ g/dl	8.0	17.2	16.7	12.0	0.54
Serum tocopherol (mg/dl)					
<i>n</i>	25	29	24	25	
Mean \pm s.d. change from baseline	0.09 \pm 0.18	0.16 \pm 0.22	0.12 \pm 0.38	0.16 \pm 0.17	0.36

Abbreviations: BSC, bovine serum concentrate; MMN, multiple micronutrients; WPC, whey protein concentrate.

^aRaw data presented; P-values derived from ANCOVA for continuous variables; logistic regression for proportions, controlling for baseline values.

^bP for MMN main effect < 0.05.

^cAnalysis based on logarithm-transformed variable.

^dP for interaction < 0.05.

treatment group in the prevalence or incidence of respiratory infections or other symptoms of morbidity.

Micronutrient status

The overall mean concentrations of both hemoglobin and serum ferritin declined during the period of supplementation (Table 6). Controlling for initial hemoglobin and other co-variables, the hemoglobin levels fell slightly less in the groups of children who received supplements that contained MMN ($P=0.05$), and a smaller proportion of the children who received MMN supplements were anemic at the end of the trial ($P<0.05$). Mean serum ferritin values declined in all groups, but this fall was significantly less in group MMN + WPC ($P=0.02$). None of the other markers of micronutrient status changed significantly in relation to treatment group, after controlling for initial micronutrient status and other covariates. In all four groups, the proportion of children with low plasma zinc and serum retinol concentrations tended to be lower at the end of the study than at the beginning.

Discussion

Dietary supplementation of Guatemalan infants with small amounts of cereal-based puddings that contained BSC, MMN, or both during periods of 2–8 months did not affect their growth velocity or rates of infection compared with those of infants who received the same preparations containing added WPC only. On the other hand, the final hemoglobin levels were greater and rates of anemia were reduced among infants who received the MMN-containing supplements. Children who received MMN + WPC also had greater final serum ferritin concentrations, although this was not true for the group that received MMN + BSC. None of the other biochemical indicators of micronutrient status differed by treatment group.

These conclusions are bolstered by several aspects of the study design, including the randomized allocation to treatment group, the masking of group assignment, the direct observation of consumption of supplements on most study days and high rates of compliance, and the careful supervision of data collectors and other quality assurance

procedures. On the other hand, the high rate of study abandonment, especially between months 6 and 8 of the intervention, reduced the initially planned sample size, hence the desired statistical power of the analyses. Nevertheless, the dropout rate did not differ by study group, so this should not have biased the results; and all analyses were completed on an intention-to-treat basis, regardless of the individual subject's duration of participation in the study. Although the major results are presented in detail only for the subset of children who remained in the study for 8 months (to assure consistency in the presentation of the different sets of results), the interpretation of the group-wise comparisons did not differ when shorter periods of observation (hence, larger numbers of children) were considered. The treatment groups were similar at baseline, the dropout rates did not differ by study group, and those who left the study early did not differ significantly from those who remained with regard to their baseline characteristics. Thus, it is reasonable to attribute any final group-wise differences to the treatments that were provided. The major reasons for dropout were study fatigue, probably because of the intensive nature and frequency of the home visits.

The study subjects were moderately stunted at baseline, and the rate of low height-for-age (z -score < -2) increased more than twofold from ~ 22 to $\sim 50\%$ during the 8-month period of observation. Contrary to our original hypotheses, the children's growth was not affected by consumption of either BSC or MMN. Previous studies found that BSC increased dietary intakes and rates of weight gain of domesticated farm animals, possibly because the IgG contained in the preparation reduced the incidence of infections (Weaver *et al.*, 1995; Pierce *et al.*, 2005). We are not aware of any relevant prior studies of BSC and growth in humans. There are several possible explanations for the differences in the results of the present study in humans compared with the earlier findings in farm animals. Firstly, the earlier studies were conducted in weaned animals, whereas most of the children in the present study were breastfed. It is conceivable that the protective effect of BSC may occur only in individuals who are not already receiving the infection-reducing benefits provided by breast milk. Secondly, it is possible that the dose employed in the present study was not sufficient to protect against infections. The dose of BSC offered in the present study provided ~ 3 – 4.5 g of protein per day, or $\sim 20\%$ of the children's total dietary protein. Studies in weaned piglets similarly used amounts of plasma protein that were $\sim 25\%$ of the animals' dietary protein, but weanling pigs typically receive about three times more total protein, so their total intake of plasma proteins would also be greater. Thus, it might be necessary to use a higher dose of BSC to achieve the same results as has been observed in farm animals. Thirdly, the specific antibodies present in the BSC may not have been appropriate to protect against the range of infections that occur most commonly in humans. Finally, there may be a different, as yet unexplained, mechanism for

the growth-promoting effect observed in farm animals, which is not operative in humans.

A number of previous studies have found increased growth among children who were supplemented either with single micronutrients, such as zinc (Brown *et al.*, 2002), or with MMN (Thu *et al.*, 1999; Rivera *et al.*, 2001; Ash *et al.*, 2003; Hop and Berger 2005). With regard to zinc, the children's low dietary zinc intakes and baseline plasma zinc concentrations suggest that they may indeed have been zinc-depleted at baseline. However, the results of previous zinc intervention trials suggest that zinc supplementation increases children's growth only when the baseline mean height-for-age of the study subjects is < -1.5 s.d. compared with international reference means (Brown *et al.*, 2002), which is just about the initial mean height-for-age of the children in the present study. Thus, the current study subjects may not have been sufficiently stunted to manifest a response to supplemental zinc.

Fewer results are available from previous growth studies of infants who received MMN supplementation, and the findings are inconsistent. Positive effects on growth were observed among infants < 12 months of age – but not among older children – in a recent study in Mexico, in which supplements were provided for 1 year (Rivera *et al.*, 2001). MMN supplementation spurred increased growth among 6- to 24-month-old Vietnamese children who were stunted at baseline, but not among those who were non-stunted, in one study (Thu *et al.*, 1999); and daily MMN supplements, but not weekly MMN supplements increased growth of young Vietnamese children in another study (Hop and Berger 2005). Among Tanzanian school children 6–11 years of age, an MMN-fortified drink increased rates of both linear growth and weight gain (Ash *et al.*, 2003). By contrast, there was no growth impact of MMN supplementation in a recent trial in Peru (Penny *et al.*, 2004) or in three sites included in a recently completed multi-center trial (López de Romaña *et al.*, 2005; Smuts *et al.*, 2005; Untoro *et al.*, 2005). The inconsistent results across these different sets of studies may relate to the age of the study subjects, their underlying nutritional status, the duration of supplementation, the composition of the supplements, or their mode of delivery (e.g., with or without meals). In the studies from Mexico and Tanzania and in one of the studies from Vietnam, the MMN supplement was given in the form of an aqueous solution. By contrast, in the present study and in the trial in Peru, the MMN supplement was provided as a cereal-based dietary supplement, which included several food ingredients. Thus, it is possible that the form of the supplement may have contributed to the observed differences among these studies.

Based on the observed variability in the children's growth velocity and the available sample size at each time point, we could have detected with a power of 0.80 group-wise differences in growth of 0.9 cm after 6 months of supplementation (or 0.5 cm if combining groups and examining only main effects of BSC or MMN) and 1.2 cm after 8 months (0.7 cm if examining only main effects). The actual shortfalls

in the children's average growth rates were 1.9 cm for the period from 6 to 12 months of age and 2.4 cm for the period of 6–14 months, compared with North American children of the same age ranges. Thus, it should have been possible to detect growth differences of this magnitude if the supplements had been able to mitigate the children's growth failure.

Neither form of supplementation (BSC or MMN) affected morbidity outcomes, although the sample size was adequate to permit detection only of fairly large differences in morbidity rates. The prevalence of diarrhea was less than expected and lower than reported in a previous study in a rural Guatemalan community in which daily supplementation with 10 mg zinc reduced the rates of diarrhea (Ruel *et al.*, 1997). While the benefit of zinc supplementation for reducing morbidity has been demonstrated in a considerable number of studies (Zinc Investigators' Collaborative Group, 1999), the effect of multiple micronutrient supplements on morbidity outcomes is still unclear. The few results available to date have been disappointing (Penny *et al.*, 2004; López de Romaña *et al.*, 2005; Smuts *et al.*, 2005; Untoro *et al.*, 2005).

Analysis of the infants' dietary data indicated low intake of several micronutrients, especially iron and zinc. By contrast, mean vitamin A intake appeared adequate because of consumption of breast milk and vitamin A-fortified foods, such as sugar, which contributed nearly 50% of the total vitamin A intake. Despite the low iron intakes, the infants had adequate iron status at baseline, presumably because they had received supplemental iron for 1–2 months before initiation of the intervention trial. Thus, we were able to examine the effect of the supplements in preventing re-emergence of iron deficiency. Although serum ferritin concentrations declined in all groups, the decrease in hemoglobin was less among those children who received MMN and the reduction in serum ferritin was less in group MMN + WPC.

The lack of response of plasma zinc concentration was unexpected because most previous studies found significant increases following zinc supplementation (Brown *et al.*, 2002). On the other hand, studies of MMN supplements have not always detected changes in plasma zinc concentration, possibly because some other component of the supplement interferes with zinc absorption or metabolism. The supplements that were distributed in the current study provided 1 RDA each of vitamins A, B₁, B₂, B₃, B₆, B₁₂, C, and D, folic acid, iron, zinc, iodine, and selenium and approximately 0.3 RDA of calcium and phosphorus. Several of the minerals, such as iron and calcium, may have limited the response to zinc (Castillo-Duran and Solomons 1991; Lind *et al.*, 2003).

In conclusion, the results of our study show that supplementation with a cereal-based pudding containing BSC and/or MMN 6 days per week during a 6- 8-month period was not effective in preventing growth stunting of young children in a peri-urban setting in Guatemala, although MMN did reduce the rates of anemia. Nutritional

supplements to enhance young children's nutritional status and growth may need to be provided in other chemical forms or delivery vehicles to be more effective or may need to be introduced during other stages of the life cycle.

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